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Composition of Cyanobacteria in Relation to Physico-Chemical Characteristics of Four Rivers in the Niger Delta

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Abstract
The composition of Cyanobacteria and physico-chemical charactaristics of Sombreiro, Orashi, New Calabar, and Bonny Rivers in the Niger Delta was studied for one year (September, 2012 - August 2013). Cyanobacteria were enumerated using the Lugol's solution method. The samples were centrifuged at 360 rpm for 15 minutes, the supernatant was carefully removed and the pellets re-suspended in 0.5 ml distilled water. The concentrated sample was placed in a haemocytometer and examined under the microscope at a magnification of 400×. The Cyanobacteria were identified and total number of species recorded using keys and checklists. Twelve species of cyanobacteria recorded in the study include *Anabaena* sp (42.34%), *Microcystis* sp (25.26%), *Cylindrospermopsis* sp (22.60%), *Nostoc* sp (2.08%), *Synechococcus* sp (1.44%), *Aphanizomenon* sp (1.44%), *Lyngbya* sp (1.17%), *Oscillatoria* sp (1.08%), *Nodularia* sp (1.08%), *Chroococcus* sp (0.81%), *Trichodesmium* sp (0.36%) and *Schizothrix* sp (0.36%). The most predominant species in this study was *Anabaena* sp (42.34%) in both the fresh and brackish water systems. The Shannon – Wiener diversity index in the four rivers ranged from 0.67 – 1.26. The population of cyanobacteria enumerated ranged from 2.0×10^3 to 1.1×10^5. The maximum value for cyanobacteria population falls within caution level and Alert 2 level of WHO (2003) which is a safe level. New Calabar River had the highest relative abundance of *Microcystis* sp, *Anabaena* sp, *Cylindrspermopsis* sp, *Lyngbya* sp, *Chroococcus* sp and *Synechococcus* sp. Correlation coefficients of cyanobacterial population and physico-chemical parameters were positive with Biochemical Oxygen demand (BOD), Chemical Oxygen Demand (COD) in Sombreiro River. Cyanobacterial counts also correlated positively with alkalinity in Orashi River and New Calabar River. The results indicate that higher organic load and more alkaline pH encourage cyanobacterial diversity.
Keywords: Cyanobacteria, Composition, Rivers, Nigeria

1.0 Introduction

Cyanobacteria or blue-green algae comprise a diverse group of prokaryotic organisms. Their long history of evolution on Earth (~3.5 billions of years) has enabled them to live in different environments and occupy distinct niches. They can be found from the poles to tropical regions, in both terrestrial and marine ecosystems (Whitton and Potts, 2000). The prominent habitats of cyanobacteria are limnic and marine environments. They flourish in water that is salty, brackish or fresh, in cold and hot springs, and in environments where no other microalgae can exist (Luuc *et al*., 1999). Similarly, many of these organisms are capable of either exploiting or modifying their habitats, to make them more suitable under environmental stress conditions, i.e. by synthesis of biologically active natural products, toxins, to eliminate competitors (Ehrenreich *et al*., 2005). If water containing high concentrations of toxic cyanobacteria or their toxins is ingested make (in drinking water or accidentally during recreation), they present a risk to human health (Bartram *et al,* 1999). Cyanobacteria are ubiquitous in surface waters worldwide and many species including *Microcystis*, *Nodularia*, *Cylindrospermopsis*, *Anabaena*, and *Aphanizomenon* are known to produce toxins (Landsberg, 2002).

Wherever conditions of temperature, light and nutrient status are conducive, surface waters (both freshwater and marine) may host increased growth of algae or cyanobacteria. Where such proliferation is dominated by a single (or a few) species, the phenomenon is referred to as an algal or cyanobacterial bloom (Bartram., *et al,* 1999). Even within a single-species bloom there may be a mixture of toxic and non-toxic strains of cyanobacteria (Sivonen and Jones, 1999). Phosphorus is the major nutrient controlling the occurrence of water blooms of cyanobacteria in many regions of the world, although nitrogen compounds are sometimes relevant in determining the amount of cyanobacteria present (Bartram *et al.*, 1999). Cyanobacterial growth is constrained by low levels of light, temperature, and nutrients (Luuc *et al*., 1999). In tropical areas, the first two of these are rarely limiting so nutrient availability is usually the key determinant of their proliferation. If cyanobacteria are present or even dominant for most of the year, the practical problems associated with high cyanobacterial biomass and the potential health threats from their toxins increase. High cyanobacterial biomass may also contribute to aesthetic problems, impair recreational use (due to surface scums and unpleasant odours), and

affect the taste of treated drinking water (Bartram *et al*., 1999).

Cyanobacteria occur in fresh, brackish and salt water bodies in Nigeria and form blooms when conditions are favourable (Gikuma-Njuru *et al*., 2005). Checklists of Cyanobacteria in some parts of Nigeria have been documented by different workers. For instance Nwankwo (1993) reported eight Cyanobacteria bloom species of coastal waters in south western Nigeria. Odokuma and Isirima (2007) identified *Anabaena* and *Microcystis* as the most predominant cyanobacteria in river water in the Niger Delta. In the North, China and Boko (2008) observed a total of thirteen species of cyanobacteria in their study in some fresh water ecosystems in the Nigerian Guinea Savannah and *Microcystis* sp was observed to be the most dominant species.

This present study was carried out to provide baseline data on the composition and abundance of cyanobacteria from four selected rivers in the in the Niger Delta, Nigeria. It will also provide a scientific basis for decision-making in the event of a cyanobacterial bloom.

Table 1. Nature of water samples and station Codes

Code	Station	Source of Sample	Nature of sample
SR1	Odhiaje	River Sombreiro	Fresh water
SR2	Ahoada	River Sombreiro	Fresh water
SR3	Agbandele	River Sombreiro	Fresh water
OR1	Mbiama	River Orashi	Fresh water
OR2	Ogbeme	River Orashi	Fresh water
OR3	Ogonokom	River Orashi	Brackish water
NCR1	Choba	River New Calabar	Brackish water
NCR2	Rumuopkarali	River New Calabar	Brackish water
NCR3	Rumuolumini	River New Calabar	Brackish water
BR1	Rumuomasi	River Bonny	Fresh water
BR2	By P.H main abattoir	River Bonny	Brackish water
BR3	Bonny waterside	River Bonny	Brackish water

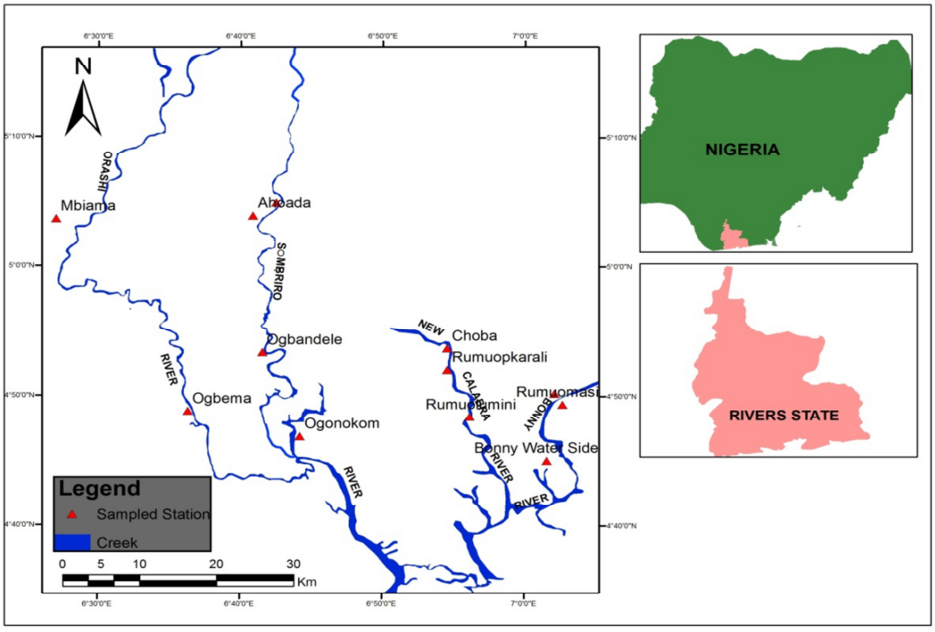

Figure 1. Map showing study area

2.0 Materials and Methods.

2.1 Study Area

The study was carried out in Sombreiro River, Orashi River, New Calabar River and Bonny River, in the Niger Delta for a period of one year (September, 2012-August, 2013). These four rivers drain the western and eastern parts of Rivers state. The area of study lies within Latitude 6°30´ and 7°10´E and Longitude 4°40´ and 5°10´N. Sombreiro River and Orashi River are distributaries of the River Niger which arises from the northern boundary of Rivers State with Imo State. Orashi River drains into Sombriero River which drains into the Atlantic Ocean.

The upper reaches of New Calabar River and Bonny River on the other hand originate from Rivers State and both rivers also drain into the Atlantic Ocean. All the rivers are lotic throughout the year. From upstream these rivers are fresh water habitats contained within the tropical rain forests while the lower reaches are within the brackish mangrove zone.

Three sampling stations each were established along the length of the four rivers on the basis of accessibility by road and in such a manner to provide an even spread for effective sampling. Several communities exist along the rivers and the people are involved in various activities such as fishing, farming, lumbering, hunting and swimming. Some of the inhabitants along the rivers use the water both for drinking and washing.

2.2 Sample collection

Surface water samples were collected monthly in each of the sampling stations shown in figure 1. The nature of the water samples and station codes employed to distinguish them are shown in Table 1. At each station, ten composite samples were collected within a 10 m radius and pooled together for physico-chemical analysis. The physico-chemical parameters investigated include hydrogen ion concentration (pH), alkalinity, phosphate, Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) using methods adapted from APHA, (1998). Samples for Biochemical Oxygen Demand (BOD) were collected with 250 ml BOD bottles, while 500 ml sterile glass jars with metal caps were used to collect water samples for the determination of the other physicochemical parameters. Brown glass bottles were used for the collection of water samples for cyanobacterial examination. 100 ml samples were fixed (1:100 ml) in Lugol solution for the identification and quantification of the Cyanobacteria. Lugol's solution is commonly used for short-term (e.g. a few months, but not possibly a year or more) storage of cyanobacteria. Lugol's solution is prepared by dissolving 20g of potassium iodide in 200ml of distilled water, mixed thoroughly and 10g of iodine crystals added (Utkilen *et al*., 1999). All samples were analysed immediately on reaching the laboratory.

2.3 Enumeration of cyanobacterial populations.

The Lugol's solution method was used for cyanobacteria enumeration. The Lugol's solution enhances sedimentation, because uptake of iodine increases the specific weight of the cells (Lawton *et al*., 1999). Twenty millilitres of sample was placed in centrifuge tube and 0.1ml of 1% hydrated aluminium potassium sulphate solution was added to enhance pelleting. The tube was sealed and centrifuged at 360 rpm for 15 minutes. The supernatant was carefully removed and the pellets re-suspended in 0.5 ml distilled water. The cyanobacteria were then counted as cells, colonies or filaments using a haemocytometer under the microscope at a magnification of 400x (Lawton *et al*., 1999). Cyanobacteria were identified using the keys and checklists of Anagnostidis and Koma'rek (1985).

2.4 Statistical Analysis.

Correlation analysis of water parameters and cyanobacterial population was done. This was to verify factors that significantly influence the abundance of cyanobacteria. Cyanobacteria diversity was assessed using Shannon – Wiener index of diversity which Ogbeibu (2005) expressed as: $H = -\Sigma$ Pi (InPi) where Pi is the proportion of species in the sample. H = Shannon – Wiener index.

3.0 Results and Discussion

The percentage abundance of cyanobacterial species in the study area is presented in Fig 2. A total of 12 Cyanobacterial species were observed in the present study. *Microcystis* sp, *Anabaena* sp, and *Cylindrospermopsis* sp occurred in all the stations. *Anabaena* sp was the most abundant cyanobacteria species (42.34%) during the study period. This was followed by *Microcystis* sp consisting of 25.26%, Cylindrospermopsis sp (22.60%), *Nostoc* sp (2.08%), *Synechococcus* sp (1.44%), *Aphanizomenon* sp (1.44%), *Lyngbya* sp (1.17%), *Oscillatoria* sp (1.08%), *Nodularia* sp (1.08%), , *Chroococcus* sp (0.81%), *Trichodesmium* sp (0.36%) and *Schizothrix* sp (0.36%). Odokuma and Isirima (2007) have reported similar observations. They observed that *Anabaena* sp was the most predominant cyanobacteria in river water samples in the Niger Delta. They also observed that *Anabena* and *Microcystis* were more predominant in the river and pond water while *Anabena* and *Cylindrospermopsis* were more predominant in ground water.

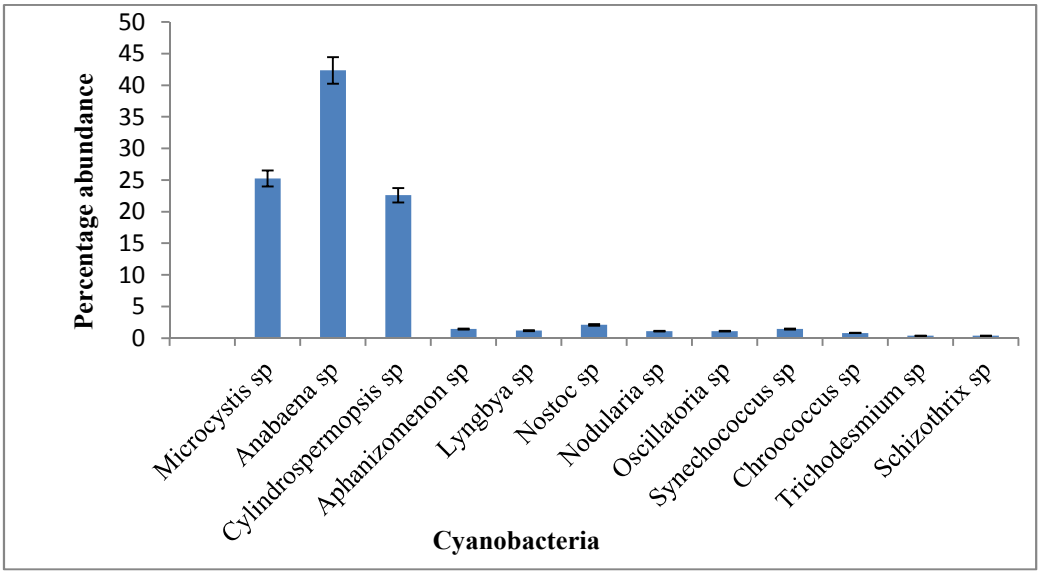

Figure 2. Group abundance of cyanobacterial species in the study area

The percentage abundance of cyanobacteria species in Sombreiro River is presented in Fig 3. A total of 7 species were recorded with *Anabaena* sp, *Microcystis* sp and *Cylindrospermopsis* sp occurring in all the stations. *Microcystis* sp was most abundant (13%) in SR1 while *Anabaena* sp was the dominant species in SR2 and SR3 with a relative abundance of 15.35% and 13% respectively. *Nostoc* sp and *Aphanizomenon* sp were observed in SR2 and SR3 while *Oscillatoria* and *Chroococcus* sp were observed only in SR3. Some of the cyanobacteria identified in this study were also observed by Ezekiel *et al*, (2011) who identified 8 species of Cyanobacteria in Sombreiro River and *Anabaena* sp was the most abundant.

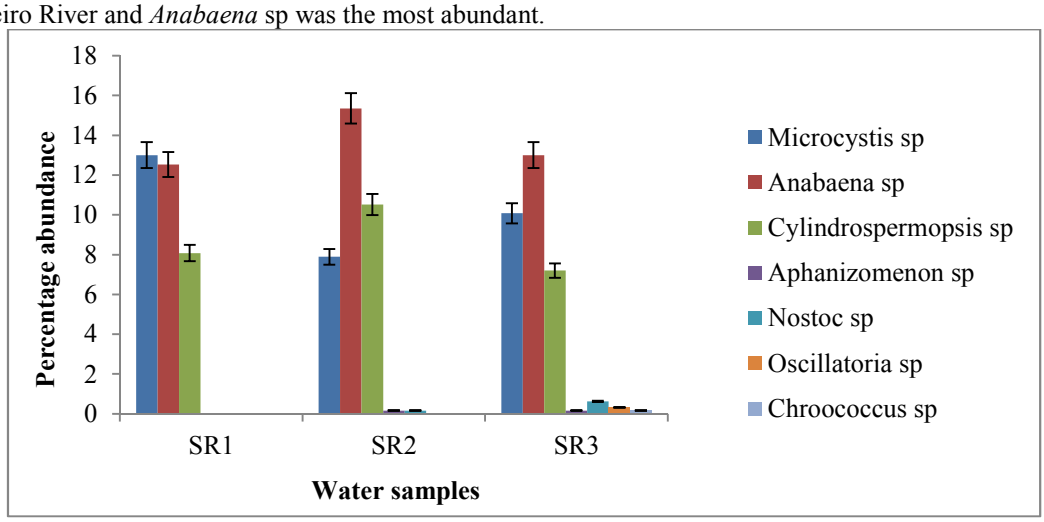

Figure 3. Percentage abundance of cyanobacterial species in Sombreiro River

The percentage abundance of cyanobacteria in Orashi River is presented in Fig 4. A total of 7 species were recorded with *Anabaena* sp, *Microcystis* sp, *Cylindrospermopsis* sp, *Aphanizomenon* sp and *Nostoc* sp occurring in all the stations. *Anabaena* sp was the most abundant in all the stations with the highest percentage abundance (17.74%) occurring in OR2. *Chroococcus* sp was observed in OR1 and OR2. *Lyngbya* sp and *Schizothrix* sp were recorded once in OR1 and OR3 respectively.

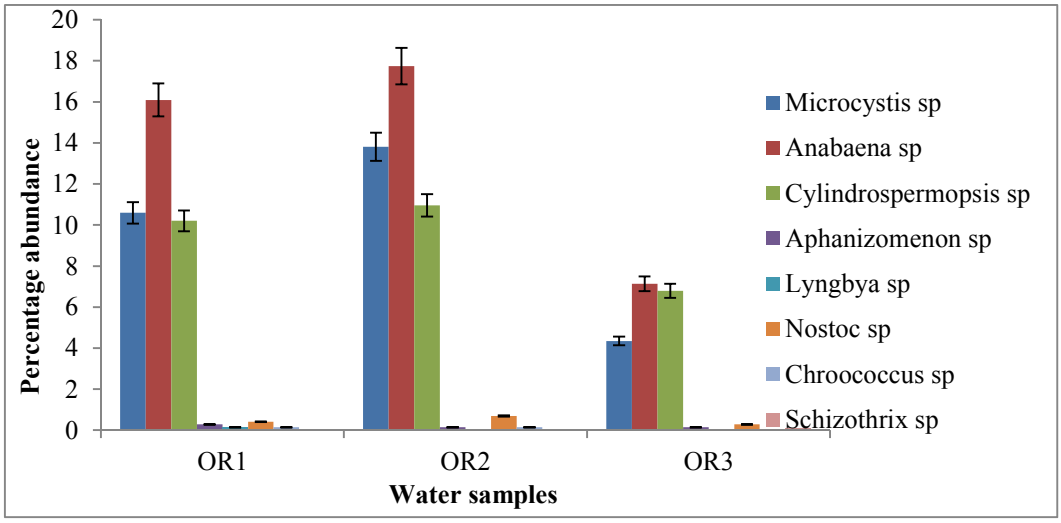

Figure 4. Percentage abundance of cyanobacterial species in Orashi River

The percentage abundance of cyanobacteria in New Calabar River is presented in Fig 5. A total of 9 species were recorded with *Anabaena* sp, *Microcystis* sp, *Cylindrospermopsis* sp, and *Lyngbya* sp occurring in all the stations. *Anabaena* sp was observed as the most abundant species in all the stations with the highest (24.81%) occurrence in NCR1. *Nostoc* sp was observed in NCR1 and NCR3. *Chroococcus* sp was observed in NCR2 and NCR3 while *Synechococcus* sp and *Trichodesmium* sp were observed once in NCR1. *Nodularia* sp was also observed once in NCR3.

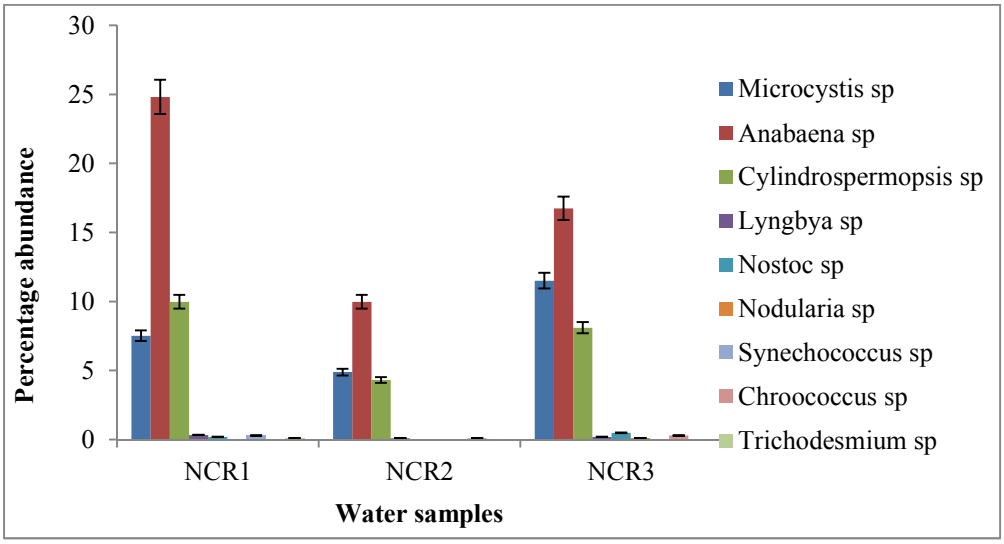

Figure 5. Percentage abundance of cyanobacterial species in New Calabar River

The percentage abundance of cyanobacteria in Bonny River is presented in Fig 6. A total of 10 species were observed with *Microcystis* sp, *Anabaena* sp, *Cylindrospermopsis* sp and *Nostoc* sp recorded in all the stations. *Anabaena* sp was the most predominant species with the highest (17.1%) occurrence in BR2. *Nodularia* sp was recorded twice in BR2 and BR3 while *Lyngbya* sp and *synechococcus* sp were recorded once in BR1. *Oscllatoria* sp, and *Chroococcus* sp were recorded in BR2 while *Aphanizomenon* sp was observed in BR3.

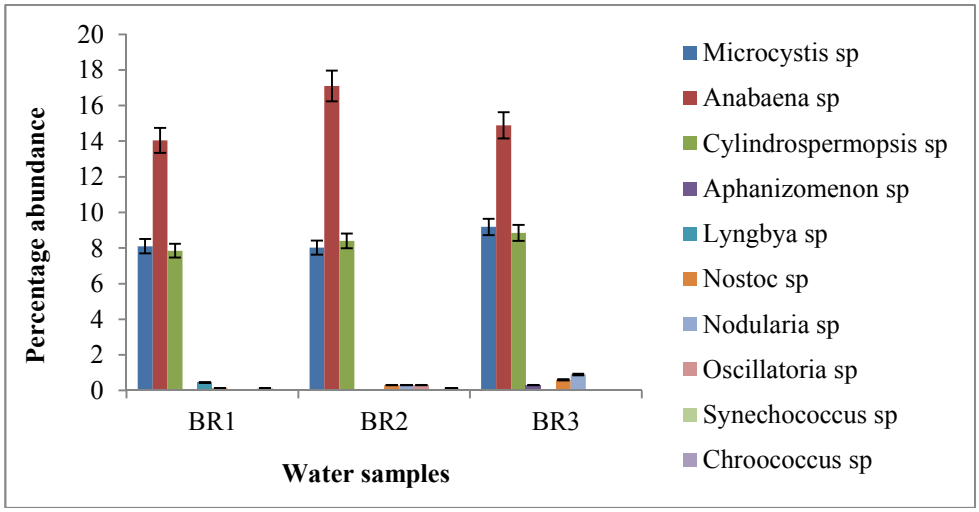

Figure 6. Percentage abundance of cyanobacterial species in Bonny River

The relative abundance of *Microcystis* sp, *Anabaena* sp, *Cylindrspermopsis* sp, *Lyngbya* sp, *Chroococcus* sp and *Synechococcus* sp in the study area are presented in Table 2. These species were identified to be most predominant in New Calabar River. *Microcystis* sp and *Chroococcus* sp were most abundant in NCR3, while *Anabaena* sp, *Cylindrspermopsis* sp, *Lyngbya* sp and *Synechococcus* sp were most abundant in NCR1. The high relative abundance of these cyanobacteria species in New Calabar River may be partly attributed to nearness to the sea and to tidal influences in the sampled stations, this bringing more nutrients to downstream sites. It may also be attributed to inputs from industrial discharges, erosional and surface run-off (Odokuma and Okpokwasili, 1993).

Table 2. Relative abundance of cyanobacteria in stations sampled in the study area

	Microcystis **sp**	***Anabaena*** **sp**	***Cylindrospermopsis*** **sp**	***Lyngbya*** **sp**	***Chroococcus*** **sp**	***Synechococcus*** **sp**
SR1	10.62	5.69	6.70	0.00	0.00	0.00
SR2	6.06	6.96	8.72	0.00	0.00	0.00
SR3	7.74	5.90	5.97	0.00	12.50	0.00
OR1	9.26	8.31	9.63	9.09	12.50	0.00
OR2	12.06	9.17	10.34	0.00	12.50	0.00
OR3	3.80	3.68	6.40	0.00	0.00	0.00
NCR1	9.50	18.54*	13.59*	36.37*	0.00	75.00*
NCR2	6.16	7.44	5.88	9.09	12.50	0.00
NCR3	14.54*	12.51	11.04	18.18	37.50*	0.00
BR1	6.50	6.65	6.80	27.27	0.00	25.00
BR2	6.42	8.10	7.27	0.00	12.50	0.00
BR3	7.34	7.05	7.66	0.00	0.00	0.00

*Stations in which the cyanobacterial genera identified were most abundant

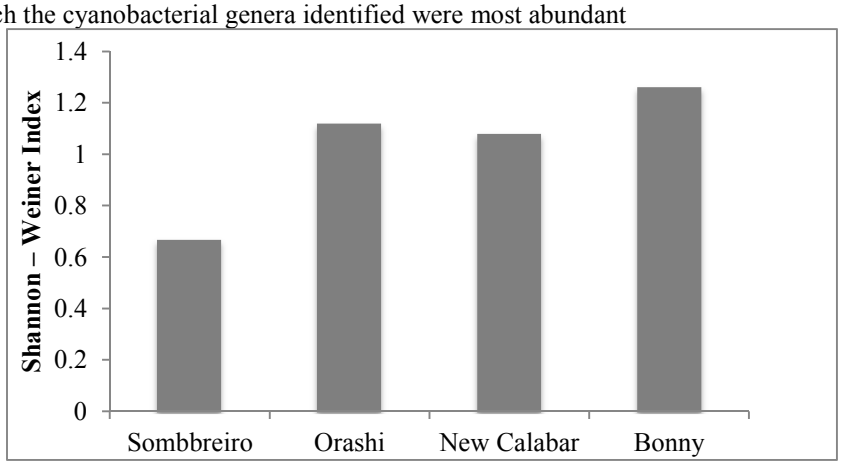

Figure 7: Shannon – Weiner Index of Cyanobacteria in the four rivers.

Cyanobacterial population ranged between 2.0×10^3 and 1.1×10^5 cells/ml in the study area. The maximum level (1.1×10^5 cells/ml) was observed in NCR1 in March. The range of the cyanobacterial population falls within caution level and Alert 2 level of WHO, (2003). At this level, monthly monitoring of cyanotoxins should be done especially in places where the river water is used for domestic consumption. The Shannon – Wiener diversity index in the four rivers ranged from 0.67 – 1.26 as shown in Fig. 7. Bonny River was noted to have higher cyanobacterial diversity than the other three rivers. Orashi River had slightly higher diversity than New Calabar River while Sombreiro River had the least diversity. Cyanobacteria are among the phytoplankton that form the base of any aquatic food chain and Organic production in the coastal ecosystem (Carol and Timothy, 1993). The Arithmetic mean of some physico-chemical parameters of the four rivers investigated is presented in Table 3.

Table 3. Arithmetic mean of physico-chemical parameters of water samples from the four rivers investigated in the study area (September 2012 - August 2013).

Property	International standard (WHO)*	Sombreiro River	Orashi River	New Calabar River	Bonny River
pH	6.5-8.5	5.40	6.11	6.25	6.9
Total Alkalinity (mg/l)	30-50	8.5	23.4	18.8	29.2
Phosphate (mg/l)	0.5	2.4	1.1	2.4	1.7
Nitrate (mg/l)	50	1.7	1.2	1.4	2.7
BOD_5 (mg/l)	4.0	18.9	22.5	23.3	42.0
COD (mg/l)	-	44.3	50.6	63.9	142.8

*Source: WHO (1993)

Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values were higher in New Calabar and Bonny Rivers than Sombreiro and Orashi Rivers. The high BOD values recorded from all the rivers might be due to untreated sewage and industrial effluents that is being discharged into the rivers contributing to higher BOD in the river systems. Bonny River had the Highest BOD and COD values, indicating a higher level of pollution than the other three rivers. This may be responsible for the higher cyanobacterial biodiversity observed in the Bonny River. Tables 4 – 7 show the correlation coefficients of cyanobacterial population and some physico-chemical parameters examined during the study. The cyanobacterial population in Sombreiro River had a positive correlation with Biochemical Oxygen demand (BOD) and Chemical Oxygen Demand (COD). However, it correlated negatively with pH. Cyanobacterial counts also correlated positively with alkalinity in Orashi River and New Calabar River. However, it correlated negatively with phosphate in all the rivers investigated.

Table 4. Correlation coefficients of Cyanobacteria and water parameters in Sombreiro River (September 2012 - August 2013)

	Cyanobacteria	BOD (mg/l)	COD (mg/l)	pH	Phosphate (mg/l)	Nitrate (mg/l)	Alkalinity (mg/l)
Cyanobacteria	1.00000						
BOD	0.61094*	1.00000					
COD	0.52516*	0.95488	1.00000				
pH	-0.57051*	0.19753	0.28071	1.00000			
Phosphate	-0.49399	-0.31042	-0.25801	0.26563	1.00000		
Nitrate	0.02444	-0.13501	-0.08449	0.07310	-0.24792	1.00000	
Alkalinity	-0.24838	0.17983	0.23373	0.64361	-0.27851	0.51736	1.00000

*Significant at $p < 0.05$

The measurement of COD is important to know the quantities of organic compounds in water. It is used to indirectly measure the amount of organic compounds in water (Kumar *et al*., 2011). The positive correlation with BOD and COD in Sombreiro River is an indication of the level of pollution in the river. It also implies that cyanobacterial population increased with an increase in the amount of organic compounds in the river.

Table 5: Correlation coefficients of Cyanobacteria and water parameters in Orashi River (September 2012 - August 2013)

	Cyanobacteria	BOD (mg/l)	COD(mg/l)	pH	Phosphate (mg/l)	Nitrate (mg/l)	Alkalinity (mg/l)
Cyanobacteria	1.00000						
BOD	-0.59741*	1.00000					
COD	-0.38097	0.77585	1.00000				
pH	-0.32083	0.58659	0.69390	1.00000			
Phosphate	-0.23305	0.09050	-0.00832	0.38175	1.00000		
Nitrate	-0.09551	-0.30108	-0.31568	0.31199	0.26762	1.00000	
Alkalinity	0.78288*	-0.52653	-0.61529	-0.63312	-0.34347	-0.22367	1.00000

*Significant at $p < 0.05$

Positive correlation between N: P and high cyanobacterial counts have been reported in river water and this highlights the importance of these nutrients for development (Barros *et al*., 1999). In natural systems, however, this correlation may not have such high relevance. A low ratio between Nitrogen and phosphorus concentration may favour the development of cyanobacteria blooms. The optimum ratio is 10 -16 molecules N: 1 molecule P (Mur *et al*., 1999). The negative correlation with phosphate recorded in this study indicates that phosphorus was not a level that will induce the growth of cyanobacteria in the four rivers. Phosphorus is an important metabolic nutrient and its availability determines productivity in natural water, even though it is dependent on pH (Samocha *et al*., 2004). Cyanobacteria have been generally reported to prefer neutral to slightly alkaline pH for optimum growth (Koushik, 1994).The pH of Bonny River was close to neutral, this may have contributed to the higher cyanobacterial diversity index of the river.

Table 6: Correlation coefficients of Cyanobacteria and water parameters in New Calabar River (September 2012 - August 2013)

	Cyanobacteria	BOD (mg/l)	COD (mg/l)	pH	Phosphate (mg/l)	Nitrate (mg/l)	Alkalinity (mg/l)
Cyanobacteria	1.00000						
BOD	0.24260	1.00000					
COD	0.29122	0.95509	1.00000				
pH	0.31753	0.13246	0.10483	1.00000			
Phosphate	-0.12642	-0.47030	-0.30803	-0.30391	1.00000		
Nitrate	0.08959	0.31329	0.26914	0.14019	-0.23359	1.00000	
Alkalinity	0.55255*	0.12457	0.15945	0.19460	0.17747	-0.46042	1.00000

*Significant at p< 0.05

Table 7: Correlation coefficients of Cyanobacteria and water parameters in Bonny River (September 2012 - August 2013)

	Cyanobacteria	BOD (mg/l)	COD (mg/l)	pH	Phosphate (mg/l)	Nitrate (mg/l)	Alkalinity (mg/l)
Cyanobacteria	1.00000						
BOD	0.17643	1.00000					
COD	0.28881	0.97489*	1.00000				
pH	0.03137	0.060643	0.02652	1.00000			
Phosphate	-0.18467	-0.012282	0.02579	-0.37752	1.00000		
Nitrate	0.05221	0.102249	0.04243	0.15263	0.43318	1.00000	
Alkalinity	-0.29284	-0.695103	-0.68039*	0.05943	0.04303	0.12147	1.00000

*Significant at p< 0.05

4.0 Conclusion.

Examination of the water samples revealed twelve different species of cyanobacteria in the study area. The cyanobacteria identified include; *Microcystis* sp, *Anabaeba* sp, *Cylindrospermopsis* sp, *Aphanizomenon* sp, *Lyngbya* sp, *Nostoc* sp, *Nodularia* sp, *Oscillatoria* sp, *Synechcoccus* sp, *Chroococcus* sp, *Trichodesmium* sp and *Schizothrix* sp. The most predominant cyanobacteria were *Anabaena* sp, *Microcystis* sp and *Cylindrospermopsis* sp. These predominant cyanobacteria are known to produce toxins. New Calabar River had a higher relative abundance of *Microcystis* sp, *Anabaena* sp, *Cylindrspermopsis* sp, *Lyngbya* sp, *Chroococcus* sp and *Synechococcus* sp. The level of cyanobacteria population in these water bodies and their potential to produce cyanotoxins implies that they are not suitable for human consumption but may be suitable for other activities. The high BOD and COD values and the near neutral pH of Bonny River may have encouraged higher cyanobacterial diversity in the river. Cyanobacterial counts had positive correlation with Biochemical Oxygen demand (BOD) and Chemical Oxygen Demand (COD) in Sombreiro River. These findings indicate that higher organic load and more alkaline pH encourage cyanobacterial diversity.

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